

# THE LANCET Infectious Diseases

## Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: van der Pluijm RW, Imwong M, Chau NH, et al. Determinants of dihydroartemisinin-piperaquine treatment failure in *Plasmodium falciparum* malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. *Lancet Infect Dis* 2019; published online July 22. [http://dx.doi.org/10.1016/S1473-3099\(19\)30391-3](http://dx.doi.org/10.1016/S1473-3099(19)30391-3).

## Appendix

Table S1. Predictors of treatment failures				
	HR (unadjusted) (95% CI)	p-value	HR (adjusted) (95% CI)	p-value
<b>Site</b>				
Ratanakiri, Cambodia (n=44)	1·000			
Binh Phuoc, Vietnam (n=60)	2·591 (1·293-5·192)	0·007		
Western Cambodia (n=17)	2·828 (1·198-6·676)	0·018		
North-eastern Thailand (n=19)	6·194 (2·821-13·600)	<0·001		
<b>Sex</b>				
Female (n=22)	1·000			
Male (n=118)	1·880 (0·857-4·123)	0·115		
<b>Age (year)</b>	0·993 (0·972-1·014)	0·492		
<b>Baseline parasite count</b>	1·000 (0·999-1·000)	0·064		
<b>Parasite clearance half-life</b>	1·166 (1·018-1·335)	0·027		
<b>Piperaquine measurable at baseline</b>				
No (n=101)	1·000			
Yes (n=30)	1·695 (0·978-2·937)	0·060		
<b>Kelch13 status</b>				
WT (n=12)	1·000		1·000	
C580Y (n=119)	3·863 (0·943-15·816)	0·060	0·632 (0·125-3·208)	0·580
<b>Plasmeprin2/3 amplification status</b>				
No amplification (n=36)	1·000		1·000	
Amplification (n=103)	4·619 (1·991-10·717)	<0·001	3·200 (1·279-8·008)	0·013
<b>Piperaquine levels at day 7 (continuous)</b>	1·003 (0·998-1·009)	0·186		
Piperaquine levels at day 7>=30ng/ml (n=72)	1·000			
Piperaquine levels at day 7<30ng/ml (n=53)	0·991 (0·581-1·690)	0·974		
<b>CRT mutation status</b>				
Other crt alleles (n=32)	1·000		1·000	
Thr93Ser (n=31)	3·880 (1·617-9·313)	0·002	4·539 (1·562-13·185)	0·005
His97Tyr (n=15)	3·017 (1·057-8·613)	0·039	3·841 (1·193-12·368)	0·024
Phe145Ile (n=16)	4·942 (1·875-13·029)	0·001	7·541 (2·396-23·732)	0·001
Ile218Phe (n=25)	3·996 (1·608-9·931)	0·003	5·099 (1·715-15·161)	0·003
Met343Ile (n=2)	5·413 (1·116-26·251)	*	5·831 (1·074-31·663)	*
Gly353Val (n=3)	8·165 (2·094-31·835)	*	10·475 (1·931-56·821)	*

\*Statistical significance not assessed due to small numbers. HR=Hazard Ratio. WT=Wild type.

Targets	Chromosome	Start	Stop	Length	Multiplex	Forward Primer Sequence	Reverse Primer Sequence
CRT 72, 74, 75, 76	Pf3D7_07_v3	403483	403687	204	1	ACACTCTTCCCTACAGACGCTTCCG ATCTTAACAGATGGCTCACGTTmUA	TCGGCATTCTGCTGAACCGCTTCCGA TCTGAGTTGGATGTTACAAAmCT
CRT 93, 97	Pf3D7_07_v3	403629	403818	189	2	ACACTCTTCCCTACAGACGCTTCCG ATCTTTGCTAAAGAACCTTAAAmCA	TCGGCATTCTGCTGAACCGCTTCCGA TCTTGGTAGGTGAATAGATTmUC
CRT 218, 220	Pf3D7_07_v3	404352	404600	248	1	ACACTCTTCCCTACAGACGCTTCCG ATCTATCTTGAAACACAAGAAGmAA	TCGGCATTCTGCTGAACCGCTTCCGA TCTATTCCTGTCATGTTGAmAA
CRT 271	Pf3D7_07_v3	404778	405026	248	2	ACACTCTTCCCTACAGACGCTTCCG ATCTTTCCAATTGTTCACTTCTmGT	TCGGCATTCTGCTGAACCGCTTCCGA TCTTATTTACCTACGACTGTmGT
CRT 326, 333	Pf3D7_07_v3	405189	405432	243	1	ACACTCTTCCCTACAGACGCTTCCG ATCTGAGCATGGTAAGAACGTTmUA	TCGGCATTCTGCTGAACCGCTTCCGA TCTCCTCTGTATGTATCACACGTmUT
CRT 356	Pf3D7_07_v3	405574	405763	189	2	ACACTCTTCCCTACAGACGCTTCCG ATCTTGTAGTTGATAACAAGGTCmCA	TCGGCATTCTGCTGAACCGCTTCCGA TCTACGTTGACCATCATAAACAmUT
CRT 371	Pf3D7_07_v3	405753	405965	212	1	ACACTCTTCCCTACAGACGCTTCCG ATCTGGTACAACGTATCATATTmUA	TCGGCATTCTGCTGAACCGCTTCCGA TCTACGAACAAGCATTGATATmUA
kelch13 BTB/POZ	Pf3D7_13_v3	1724912	1725156	244	1	ACACTCTTCCCTACAGACGCTTCCG ATCTATGAATTAGAACCTGCCAmUT	TCGGCATTCTGCTGAACCGCTTCCGA TCTTCCATATGCCATTAGAACGmCT
kelch13 BTB/POZ	Pf3D7_13_v3	1725070	1725319	249	2	ACACTCTTCCCTACAGACGCTTCCG ATCTCATTATCAATACCTCCAACAmAC	TGGCATTCTGCTGAACCGCTTCCGA TCTATCGTATGAAAGCATGGGmAG
kelch13 BTB/POZ	Pf3D7_13_v3	1725261	1725475	214	1	ACACTCTTCCCTACAGACGCTTCCG ATCTCATAGCTGATGATCTAGGmGG	TGGCATTCTGCTGAACCGCTTCCGA TCTCTGAGGTGTATGATCGTTAmAG
kelch13 BTB/POZ	Pf3D7_13_v3	1725428	1725657	229	2	ACACTCTTCCCTACAGACGCTTCCG ATCTAATTACTGAAACATACCATmAC	TGGCATTCTGCTGAACCGCTTCCGA TCTTATAGGTGGATTGATGGGmUA
kelch13 BTB/POZ	Pf3D7_13_v3	1725566	1725814	248	1	ACACTCTTCCCTACAGACGCTTCCG ATCTTAGACATAGGTGTACACATAmCG	TGGCATTCTGCTGAACCGCTTCCGA TCTTCTTAGATAGGGATAGTGAGmUT
kelch13 BTB/POZ	Pf3D7_13_v3	1725746	1725980	234	2	ACACTCTTCCCTACAGACGCTTCCG ATCTGGGTATAGTTAACGGATTmCT	TGGCATTCTGCTGAACCGCTTCCGA TCTAAAATTGTTGATGCAAATATmUG
Plasmepsin 2/3 breakpoint	Pf3D7_14_v3	298737	289836	var	2	ACACTCTTCCCTACAGACGCTTCCG ATCTCTAGGTGACCCATTATGmAG	TGGCATTCTGCTGAACCGCTTCCGA TCTTAGCTTAGCATCATTCAmCG

Table S2 – Amplicon sequencing primers used to genotype variations in the *crt*, *kelch13* and *plasmepsin2/3* genes.

In order avoid amplicon overlapping, two different multiplexes were designed. For each amplicon, we show, from left to right: the target variants located within the amplicon, the chromosome, start and end location of the amplicon (using the Pf3D7\_v3 reference); the length of the amplicon; the multiplex within which the amplicon was implemented; and the forward and reverse primer sequences.

CRT Haplotype	Counts		Allele at individual positions of the <i>crt</i> gene																
	TRACI	TRACII	72-76	220	271	371	326	356	93	97	145	218	343	353	144	148	194	333	
Wild-type (3D7-like)	9	0	CVMNK	A	Q	R	N	I	T	H	F	I	M	G	A	L	I	T	
CVIET	38	0	CVIET	S	E	I	N	I	T	H	F	I	M	G	A	L	I	T	
CVIET+Ile194Thr	10	0	CVIET	S	E	I	N	I	T	H	F	I	M	G	A	L	T/-	T	
CVIET+Ile356Thr	1	0	CVIET	S	E	I	N	T	T	H	F	I	M	G	A	L	I	T	
CVIET+N326S+356Thr	191	50	CVIET	S	E	I	S	T	T	H	F	I	M	G	A	L	I/-	T	
Thr93Ser mutation	0	82	CVIET	S	E	I	S	T	S	H	F	I	M	G	A	L	I	T	
His97Tyr mutation	10	69	CVIET	S	E	I	S	T	T	Y	F	I	M	G	A	L	I	T	
Phe145Ile mutation	0	35	CVIET	S	E	I	S	T	T	H	I	I	M	G	A	L	I	T	
Ile218Phe mutation	6	48	CVIET	S	E	I	S	T	T	H	F	F	M	G	A	L	I	T	
Met343L mutation	2	0	CVIET	S	E	I	S	T	T	H	F	I	L	G	A	L	I	T	
Met343Ile mutation	0	10	CVIET	S	E	I	S	T	T	H	F	I	I	G	A	L	I	T	
Gly353Val mutation	8	29	CVIET	S	E	I	S	T	T	H	F	I	M	V	A	L	I	T	
CVIDT+Thr333Ser	49	4	CVIDT	S	E	R	N	I	T	H	F	I	M	G	F	I	T/-	S	
CVIDT+Arg371Ile	1	0	CVIDT	S	E	I	N	I	T	H	F	I	M	G	F	I	T	T	
Heterozygote infections	49	50																	
Missingness at position 93, 97, 145, 218, 343 or 353	48	7																	
Haplotyping not possible	6	47																	

Table S3 – Prevalence of *crt* gene haplotypes in TRACI (2011-2013) and TRACII (2015-2018).

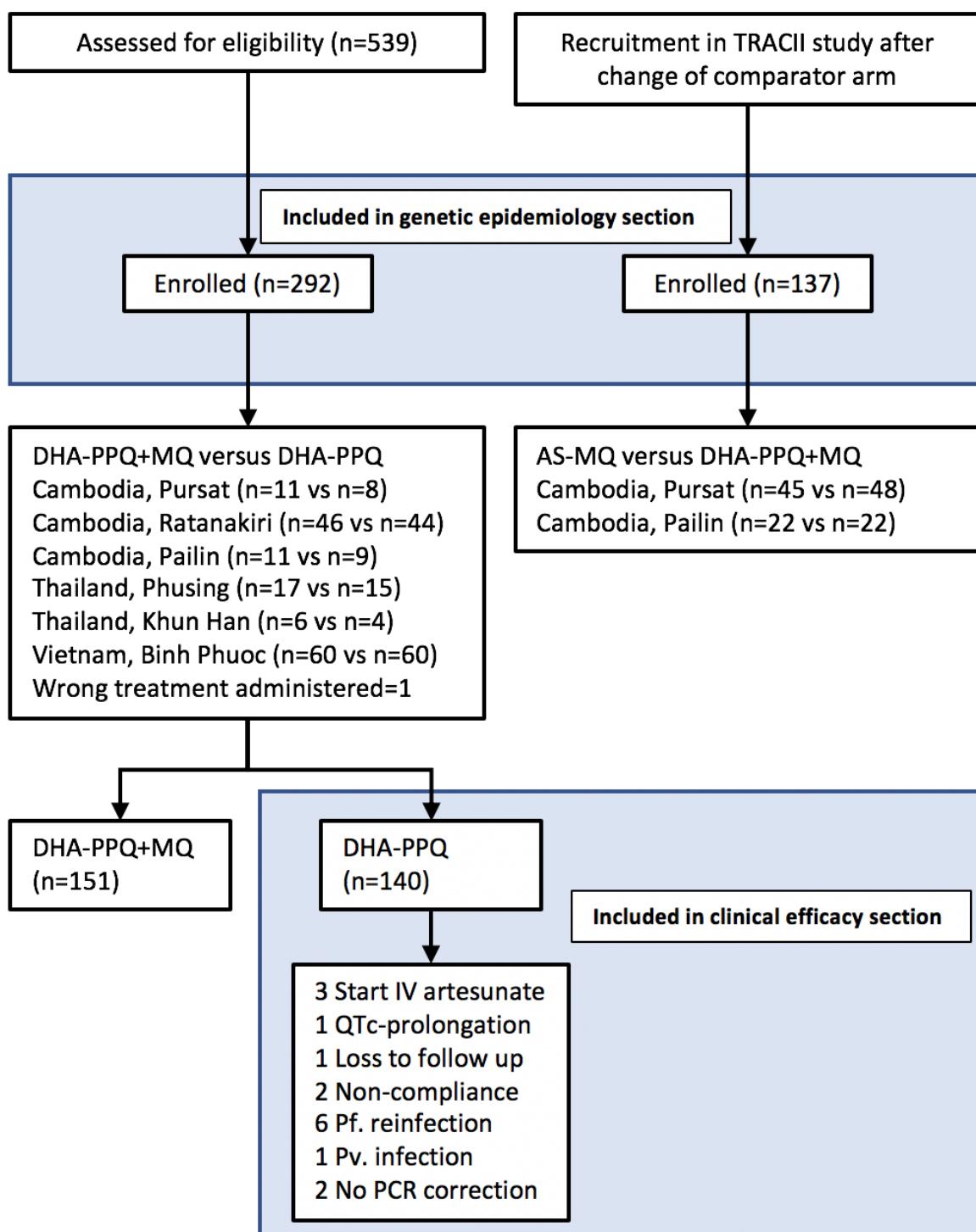


Figure S1. Simplified flow diagram of the TRACII study. The two panels indicate which patients have been included in the different sections that are presented in this manuscript.

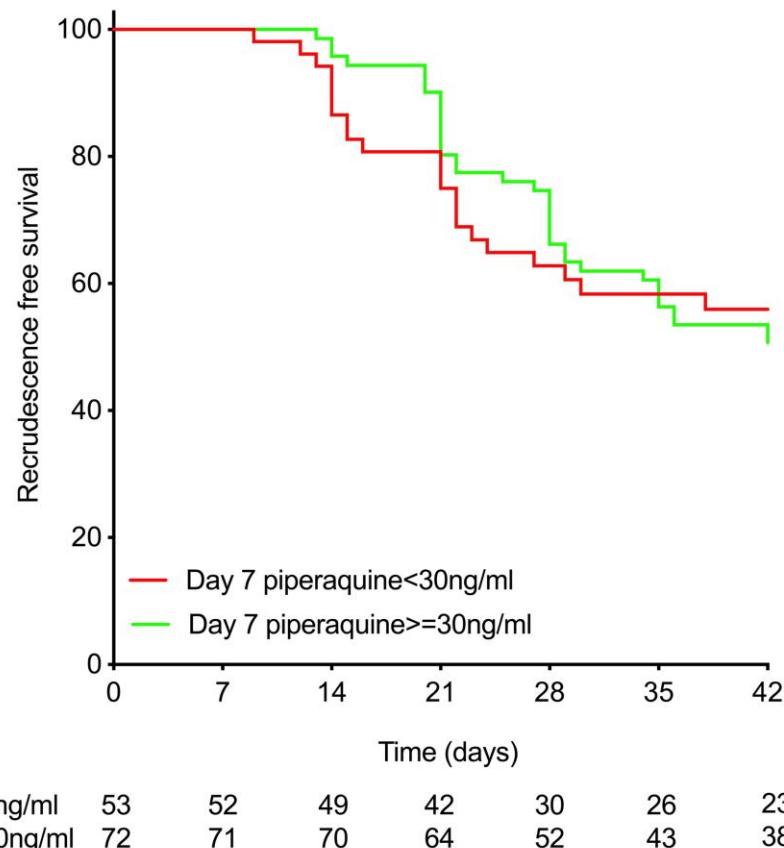


Figure S2. Kaplan-Meier survival curves describing PCR corrected efficacy of dihydroartemisin-piperaquine by day 7 piperaquine concentrations.

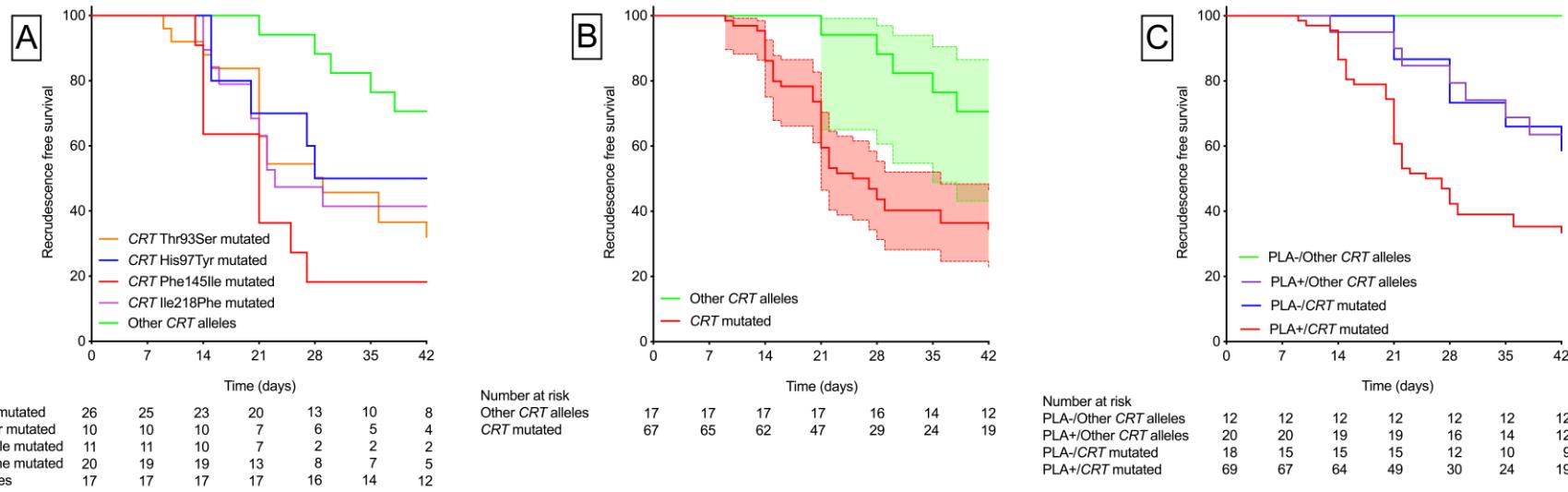


Figure S3. Kaplan-Meier survival curves describing PCR corrected efficacy of dihydroartemisinin-piperaquine by *crt* mutation status (for the subgroup of parasites bearing a *Kelch13* Cys580Tyr mutation and *Plasmepsin2/3* amplification) (Panel A and B) and a combination of *Plasmepsin2/3* amplification and *crt* mutation status (Panel C). Panel A/B/C: ‘Other *crt* alleles’ indicates parasites carrying no mutations at position 93, 97 and 145, 218, 343 and 353 of the *crt* gene. Panel B: Shaded areas indicate 95 % confidence intervals. Panel C: **PLA+** indicates parasites carrying a *Plasmepsin2/3* amplification. **PLA-** indicates parasites carrying a single copy of *Plasmepsin2/3*. Panel B/C: Dotted lines indicated 95 % confidence intervals.

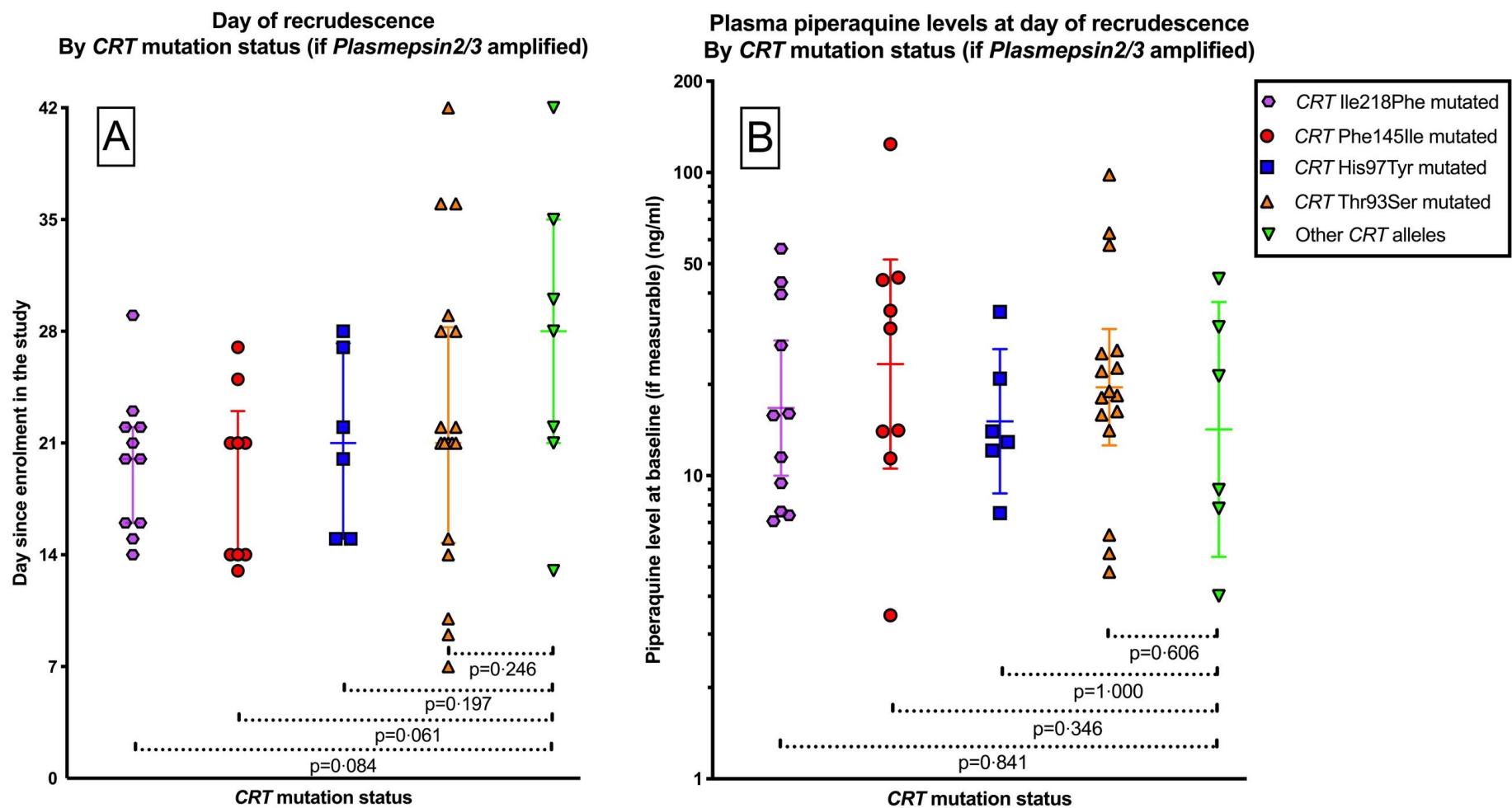
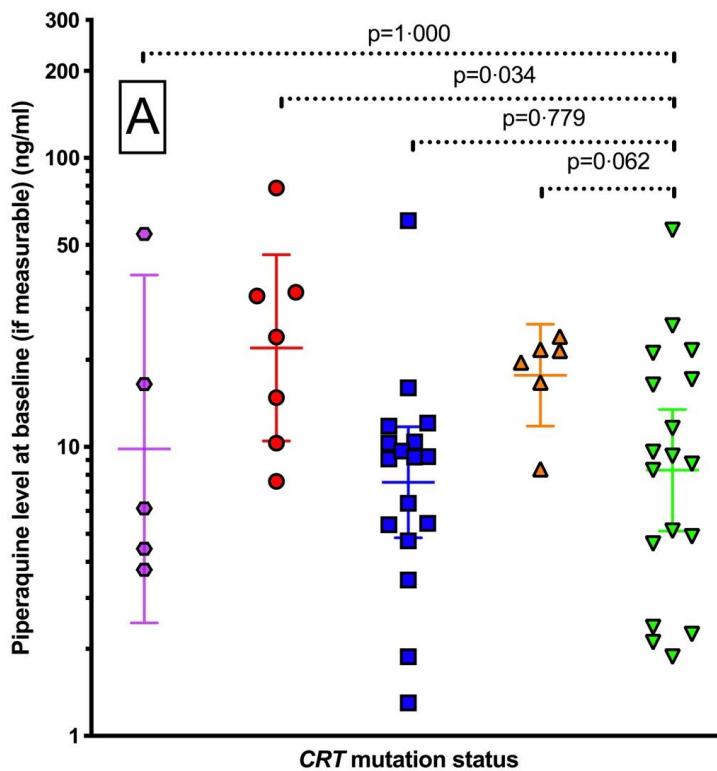


Figure S4. Day of recrudescence (Panel A) and piperaquine levels at day of recrudescence (Panel B) by *crt* mutation status for subgroup of parasites with a *Plasmepsin2/3* amplification. Panel A: Bars indicate median and interquartile ranges. Panel B: Bars indicate geometric mean and 95% confidence intervals.

**Plasma piperaquine levels at baseline (if measurable)  
By *CRT* mutation status (if *Plasmepsin2/3* amplified)**



**Plasma piperaquine levels at day 7  
By *CRT* mutation status (if *Plasmepsin2/3* amplified)**

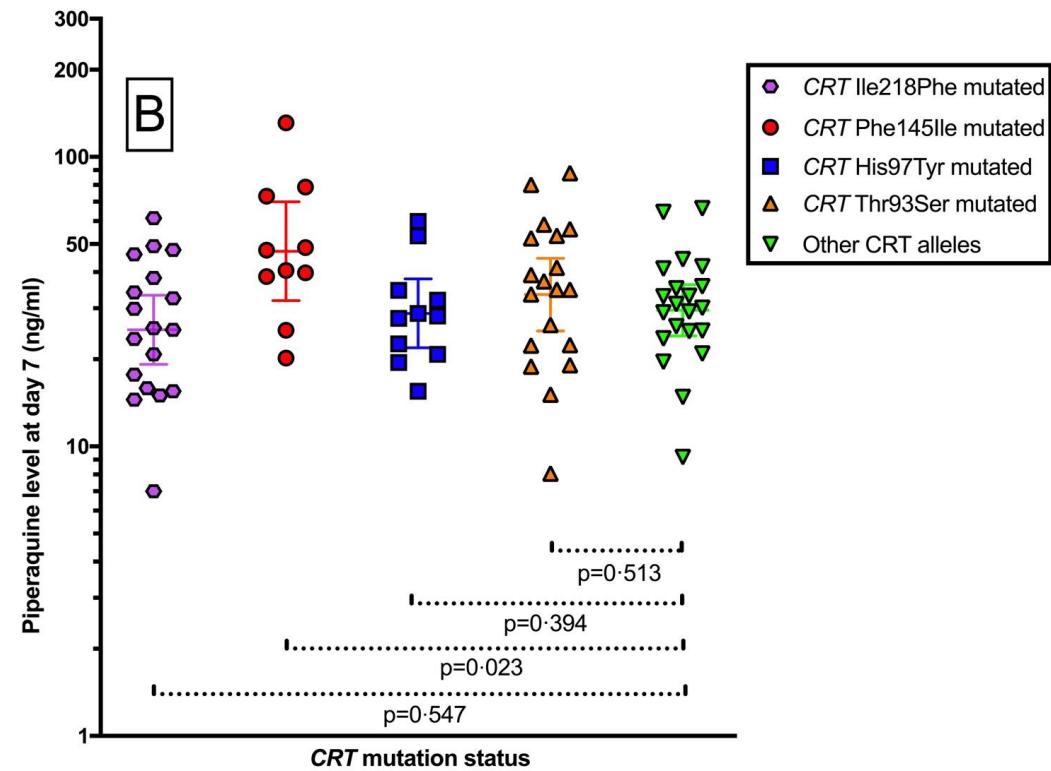


Figure S5. Baseline piperaquine levels (Panel A) and piperaquine levels at day 7 (Panel B) by *crt* mutation status for subgroup of parasites with a *Plasmepsin2/3* amplification. Bars indicate geometric mean and 95% confidence intervals.

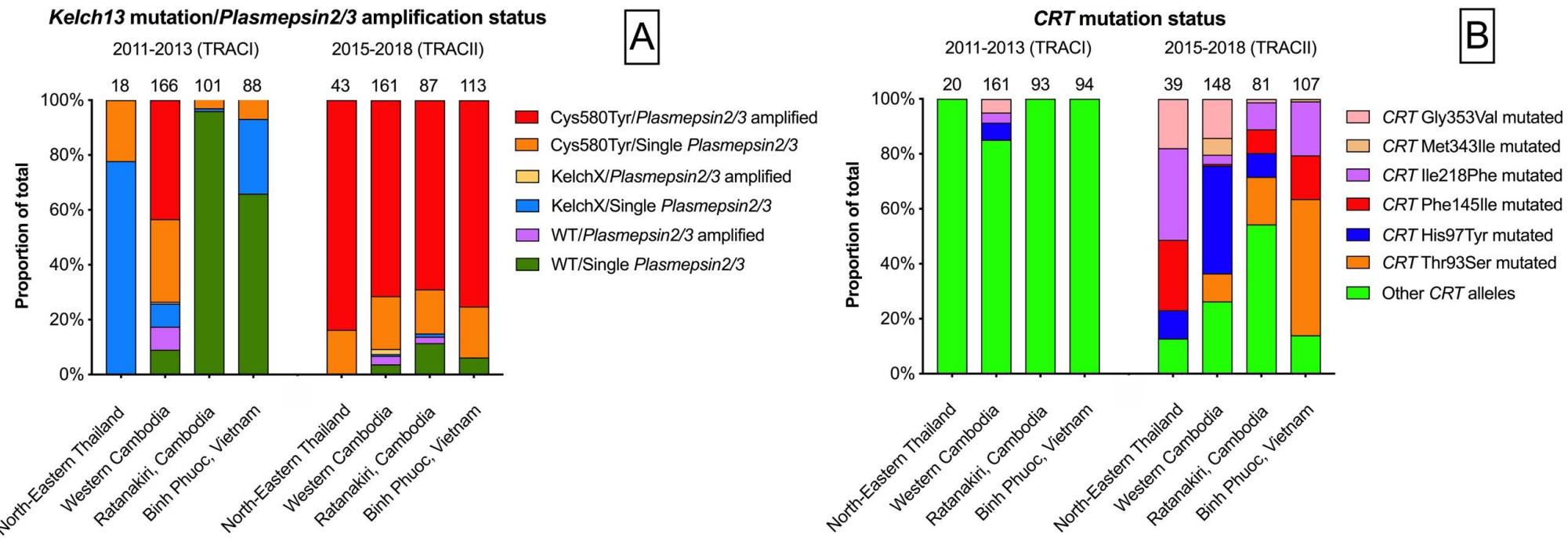


Figure S6. Frequencies of combinations for genetic markers relevant to resistance to artemisinins and piperaquine at the four sites/regions in TRACI (2011-2013) and TRACII (2015-2018). Panel A: **Cys580Tyr, KelchX and WT** indicate a *Kelch13* Cys580Tyr mutation, a *Kelch13* other than Cys580Tyr and *Kelch13* wild-type, respectively. **'Plasmepsin2/3 amplified'** and **'Single Plasmepsin2/3'** indicate parasites with or without a *Plasmepsin2/3* amplification, respectively. Panel B: Prevalence of **crt Thr93Ser, His97Tyr, Phe145Ile, Ile218Phe, Met343Ile and Gly353Val** mutations. **'Other CRT alleles'** indicates parasites carrying no mutations at position 93, 97 and 145, 218, 343 and 353 of the *crt* gene.

### **Kelch13 mutation, Plasmepsin2/3 amplification and CRT mutation status**

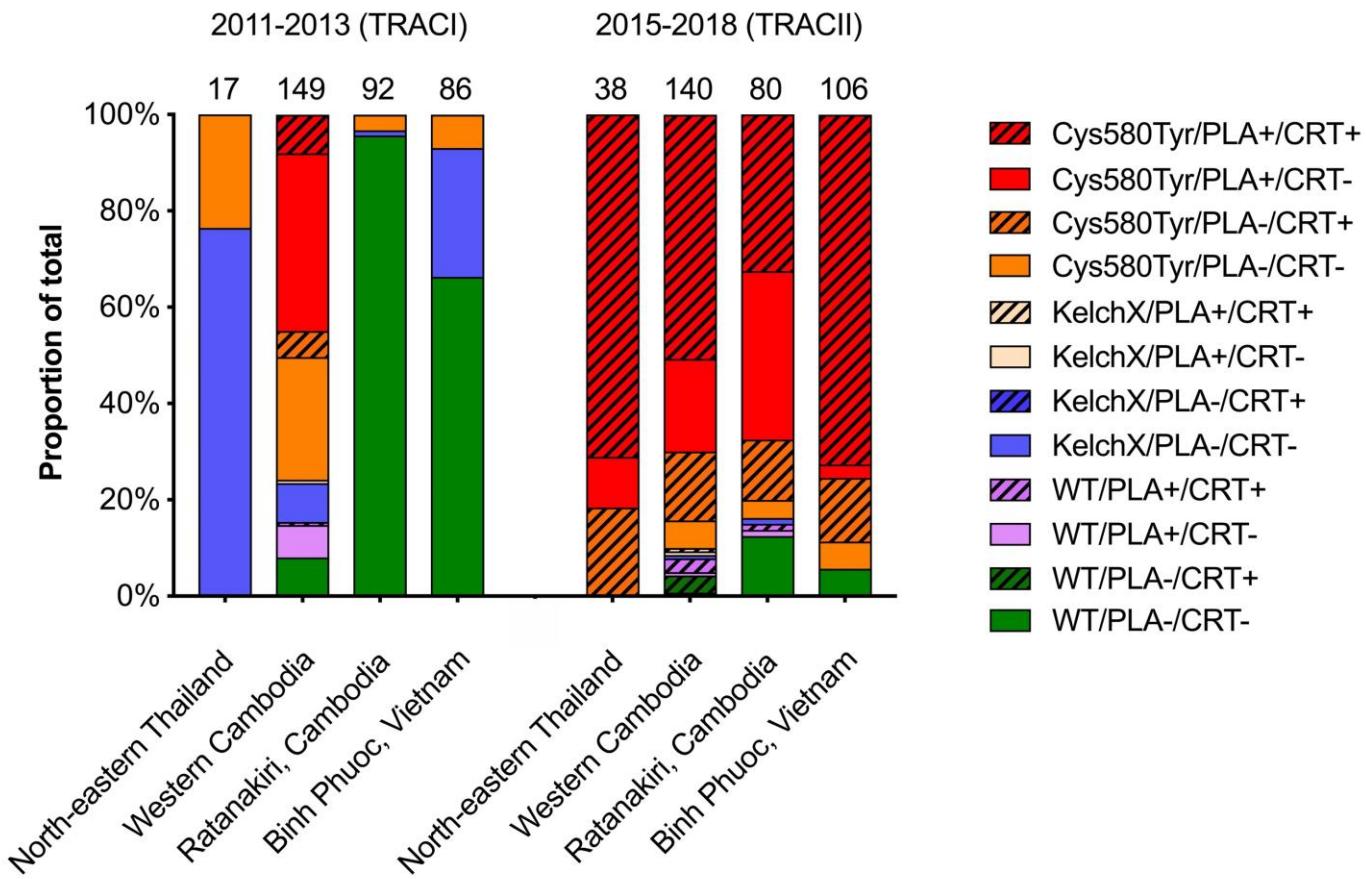


Figure S7. Frequencies of combinations for genetic markers related to resistance to artemisinins and piperaquine in TRACI (2011-2013) and TRACII (2015-2018). **Cys580Tyr**, **KelchX** and **WT** indicate a *Kelch13* Cys580Tyr mutation, a *Kelch13* other than Cys580Tyr and *Kelch13* wild-type, respectively. ‘PLA+’ and ‘PLA-’ indicate parasites with or without a *Plasmepsin2/3* amplification, respectively. ‘CRT+’ indicate parasites with one of the *crt Thr93Ser*, *His97Tyr*, *Phe145Ile*, *Ile218Phe*, *Met343Ile* and *Gly353Val* mutations whereas ‘CRT-’ identifies parasites without of one of these mutations.

**Details on medication used in trial:**

**DHA-piperaquine dosing schedule (administered at H0, H24 and H48)**

DHA-piperaquine	
Weight (kilogram)	Tablets/day (40/320 mg/tablet)
5 – 7·9	0·5
8 - 10·9	0·75
11 – 16·9	1
17 – 24·9	1·5
25 - 35·9	2
36 - 59·9	3
60 - 80·9	4
81 and more	5

**DHA-piperaquine formulations and sourcing**

Vietnam:	Arterakine	Drug Company Central 1, Vietnam	Batch: 12010/13002
Cambodia:	D-Artepp	Guilin Pharmaceutical, China	Batch: SQ150501
Thailand:	D-Artepp	Guilin Pharmaceutical, China	Batch: SQ150501/SQ160438

**Primaquine treatment schedule (administered at H24)**

Primaquine	
Weight (kilogram)	Tablet/day (15 mg/tablet)
<25	0·25
25-50	0·50
>50	1·00

**Primaquine formulations and sourcing**

Vietnam:	Primaquine	Danapha, Vietnam	Batch: 10815
Thailand:	Primaquine	GPO, Thailand	Batch: L580200/L580466
Cambodia:	Primaquine	GPO, Thailand	Batch: 64329

**Mefloquine dosing schedule (administered at H0, H24 and H48)**

<b>Mefloquine</b>	
<b>Weight (kilogram)</b>	<b>Milliliter/day (50mg/ml)</b>
<b>5 - 5.9</b>	<b>0.8</b>
<b>6 - 6.9</b>	<b>1</b>
<b>7 - 7.9</b>	<b>1.2</b>
<b>8 - 8.9</b>	<b>1.3</b>
<b>9 - 9.9</b>	<b>1.5</b>
<b>10 - 10.9</b>	<b>1.7</b>
<b>11 - 11.9</b>	<b>1.8</b>
<b>Weight (kilogram)</b>	<b>Tablet/day (250 mg/tablet)</b>
<b>12 - 16.9</b>	<b>0.5</b>
<b>17 - 23.9</b>	<b>0.75</b>
<b>24 - 24.9</b>	<b>1</b>
<b>25 - 33.9</b>	<b>1</b>
<b>34 - 35.9</b>	<b>1.25</b>
<b>36 - 43.9</b>	<b>1.25</b>
<b>44 - 48.9</b>	<b>1.5</b>
<b>49 - 53.9</b>	<b>1.75</b>
<b>54 - 59.9</b>	<b>2</b>
<b>60 - 63.9</b>	<b>2</b>
<b>64 - 71.9</b>	<b>2.25</b>
<b>72 - 77.9</b>	<b>2.5</b>
<b>78 - 80.9</b>	<b>2.75</b>
<b>81 and more</b>	<b>2.75</b>

**Mefloquine:**

Note: For younger children ( $\leq 11$  kg), mefloquine can be given by dissolving in water or other beverage (e.g. fruit juice) and a suspension is made by allowing 1 tablet to dissolve in 5ml (1ml=50mg)

**Mefloquine formulations and sourcing**

Vietnam:	Lariam	Roche Pharmaceuticals, Switzerland	Batch: B1220B02
Cambodia:	Lariam	Roche Pharmaceuticals, Switzerland	Batch: B1218B02
Thailand:	Mequin	Atlantic Laboratories Ltd, Thailand	Batch: 140130

**Treatment of recurrent infections**

Cambodia/Thailand    Artesunate+atovaquone-proguanil for 3 days

**Artesunate dosing schedule**

<b>Artesunate</b>			
Weight (kilogram)	Tablets/day (50mg/tablet)	Weight (kilogram)	Tablets/day (50mg/tablet)
11-14·9	1	43-45·9	3·5
15-16·9	1·25	46-48·9	3·75
17-20·9	1·5	49-51·9	4
21-23·9	1·75	52-54·9	4·25
24-26·9	2	55-57·9	4·5
27-29·9	2·25	58-60·9	4·75
30-32·9	2·5	61-64·9	5
33-35·9	2·75	65-67·9	5·25
36-39·9	3	68 and above	5·5
40-42·9	3·25		

**Artesunate formulation and sourcing**

Cambodia    Artesunate    Guilin Pharmaceutical, China    Batch: B1228B07

Thailand    Artesunate    Guilin Pharmaceutical, China    Batch: AS140807

**Atovaquone-proguanil dosing schedule**

<b>Atovaquone-proguanil</b>	
Weight (kilogram)	Tablets/day (250-100 mg/tablet)
11-20·9 kg	1
21 – 30·9	2
31 – 39·9	3
40 and above	4

**Atovaquone-proguanil formulation and sourcing**

Cambodia    Malanil (250-100 mg)    GSK, United Kingdom    Batch: 2G005

Thailand    Malanil (250-100 mg)    GSK, United Kingdom    Batch: 2G005/C3001

**Vietnam**                   **Quinine+doxycycline for 7 days**

**Quinine dosing schedule**

Quinine (30 mg/kg/day t.i.d.)

**Quinine formulation and sourcing**

Vietnam:                   Quinine                   GPO, Thailand                   Batch: F571314

**Doxycycline dosing schedule**

Doxycycline (3mg/kg/day q.d)

**Doxycycline formulation and sourcing**

Vietnam:                   Doxycycline                   STADA-VN, Vietnam                   Batch: 051215